

A COEUR cohort study of SATB2 expression and its prognostic value in ovarian endometrioid carcinoma

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Abstract

The aim of this study was to describe the expression of special AT-rich sequence-binding protein 2 (SATB2) in ovarian endometrioid carcinoma (EC). SATB2 is a nuclear matrix-associated transcription factor that is associated with abnormal expression in certain cancers but has not been reported for ovarian carcinoma. SATB2 mRNA and protein expression was first assessed in a pilot cohort of 26 samples by Affymetrix microarray and by routine immunohistochemistry on a small tissue microarray. A large multicenter validation cohort representing the well-characterized cases of 235 ovarian EC from the Canadian Ovarian Experimental Unified Resource (COEUR) was then used to validate this result and to assess the prognostic impact of SATB2 expression. SATB2 staining was scored as negative, weak, moderate, and strong intensity, and by percentage of stained cells. No SATB2 expression was observed in clear cell carcinomas but 10% ($n = 3$) of the ECs in the pilot cohort showed SATB2 expression. In the validation cohort, strong expression was observed in 11% of ECs, while weak or moderate expression levels were detected in 12% of cases. Evaluation of SATB2 expression with clinicopathological parameters revealed an association with patient age and Federation International of Gynecology and Obstetrics grade but not with disease stage or postoperative residual disease. Any expression of SATB2, independent of intensity, was also associated with longer survival and improved progression-free survival with hazard ratio (HR) = 0.14 (95% CI 0.03–0.56) and HR = 0.16 (95% CI 0.02–1.24) respectively. A greater beneficial effect was observed in patients with stage III/IV disease compared to patients with stage I/II disease. Furthermore, direct comparison of SATB2 with other reported prognostic biomarkers such as progesterone receptor, CDX2 and β -catenin within this cohort showed that SATB2 had the strongest association with survival. Given the current lack of accurate prognostic factors for these patients, SATB2 has promising clinical utility and warrants further study.

Keywords: survival; disease progression; progression; special AT-rich sequence-binding protein 2; progesterone receptor; CDX2; β -catenin

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Introduction

Ovarian carcinoma (OC) is the leading cause of mortality from gynecologic malignancy in the Western world and is represented by five histotypes with different molecular characteristics and clinical behaviors [1,2]. As the predominant histotype, high-grade serous carcinoma (HGSC) represents 60% of all epithelial

OCs, and its molecular nature is well characterized compared to the other histotypes. Historically, endometrioid carcinomas (ECs) account for up to 20% of OC but according to recent modern classification the frequency is 11% [2–4]. EC often presents at an early stage and is more frequently associated with endometriosis. EC is characterized by *PTEN* and β -catenin (*CTNBI*) mutations, the latter leading to positive

nuclear immunohistochemical staining [5,6]. EC is also associated with frequent diffuse expression of progesterone receptor (PR) [7] and mismatch repair deficiency in 13% and *POLE* exonuclease domain mutations in 5% of cases [8–13]. When comparing survival by histotype, HGSC patients show the worst outcome, whereas EC patients have the best prognosis, particularly at stage III of the disease, with 75–85% overall survival (OS) [2,14]. However, the survival rate of EC drops to 25–45% at an advanced stage of the disease [2,14]. Clinical variables, such as stage and residual disease, are helpful to establish the management of patients but are limited for those without residual disease. Additionally, even if it is clear that stage IA patients do not need chemotherapy, the exact threshold when chemotherapy should be given is unclear, thereby emphasizing the need for prognostic markers. In contrast, more than half of advanced stage patients will have disease progression, and limited targeted therapeutic options are available. In addition, the low frequency of ovarian EC renders this histotype difficult to study and, therefore, under-characterized. A better understanding of EC disease is needed to develop therapeutic strategies and reliable biomarkers to assist the management of these patients.

Recently, the expression of special AT-rich sequence-binding protein 2 (SATB2) has been described in the morular metaplasia associated with the endometrioid histotype of endometrial and OCs [15]. Morular metaplasia is characterized by positive immunostaining of caudal-related homeobox transcription factor 2 (CDX2) and nuclear β -catenin expression [16,17]. SATB2 is involved in chromatin organization as a nuclear matrix-associated transcription factor by binding to the nuclear matrix attachment region (MAR) [18,19]. MARs are AT-rich DNA sequences that allow MAR-binding proteins to interact with histone acetylases and deacetylases on multiple genes and specific metastasis-associated proteins to regulate epigenetic signals and chromatin remodeling. SATB2 is generally expressed in glandular cells of the lower gastrointestinal tract and in neuronal cells of the hippocampus and cerebral cortex. Expression of SATB2 has been observed in gastrointestinal cancer [20–24], renal cell carcinoma [25,26], breast cancer [27] and endometrial carcinoma [15], but rarely in OC [22,23,28,29]. For this reason, SATB2 is commonly used in pathology to discriminate primary mucinous OC from gastrointestinal metastasis to the ovary. In renal cell carcinoma and colorectal carcinoma (CRC), SATB2 expression is associated with a better outcome for patients (reviewed in [30,31]). However, in other cancers, including lung, pancreatic carcinoma and

breast cancer, SATB2 has been linked with increased tumor aggressivity (review [30,31]).

The aim of this study was to examine the expression of SATB2 in ovarian EC and its potential association with patient outcome. For this purpose, we first used a small set of samples to screen for the expression of SATB2 in OC, and then we validated the observed data in the large Canadian Ovarian Experimental Unified Resource (COEUR) cohort containing 235 ECs that have been rigorously reviewed for histotype diagnosis with a marker-assisted pathological process as previously described [32].

Methods

Patients and tissue specimens

All biobanks received ethics approval from their local review boards to collect and share samples and clinical data. The central activities of the Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), which included the collection of the COEUR repository samples and data, received local ethics approval from the Comité d'éthique de la recherche du Centre hospitalier de l'Université de Montréal (CHUM) (project reference: 2010-3552, CE 09.141 – BSP; CA). The biomarker study was also approved by the CHUM ethics committee (project reference: 2015-5927, CE 14.382; CA).

For the microarray study, RNA was purified from samples collected between 1995 and 2001 at the Hôpital Notre Dame (CHUM, Montreal, Canada). Some samples were excluded based on inappropriate histotype, incomplete follow-up, pre-operative chemotherapy or insufficient material. Less than 10% were excluded due to RNA quality, which was independent of the age of the sample. From 59 cases, only 35 produced useful chip data for analysis. Of the 35 cases, 26 samples matched the eligibility criteria for this study. Eligibility criteria for inclusion were epithelial OC tissue, no borderline tumors and ovaries as the primary tissue site.

For the tissue microarrays (TMAs), three sets of retrospective tissue cohorts were used. The first two cohorts were from tumor samples collected and banked from presumed endometrioid and clear cell carcinomas from patients undergoing surgery within the Division of Gynecologic Oncology at the CHUM from 1991 to 2009. A gynecologic oncologist scored tumor stage and residual disease according to criteria from the Federation International of Gynecology and Obstetrics (FIGO). The pilot EC TMA was composed

of 44 OC samples of which only 30 were confirmed as ovarian EC. The pilot clear cell TMA was composed of 40 clear cell carcinoma samples. The third cohort was provided by the COEUR as a TMA containing 247 OCs from a retrospective collection assembled from 12 Canadian institutions. The COEUR selection criteria, specimens and clinical data collection process have been previously described [14,33]. The histotype of formalin-fixed paraffin-embedded (FFPE) samples was reviewed by a central pathologist at the CRCHUM and confirmed on the constructed TMA by a second pathologist using the COSPv3 8-marker panel [32]. From the 247 cases of the TMA, 235 were confirmed as EC. Stage was categorized as early (stage I and II) or advanced (stage III and IV). Disease-specific survival (DSS) of patients was calculated from the time of diagnosis until death from ovarian cancer or last follow-up. OS of patients was calculated from the time of diagnosis until death from any cause or last follow-up. Progression time was calculated from first date of treatment to date of first disease progression as defined according to the Gynecologic Cancer Inter-Group (GCIG): the earliest date between CA125 rise and objective clinical disease progression. The characteristics of the tumors and patient outcome for the COEUR cohort are summarized in Table 1.

RNA extraction and microarray analysis

Total RNA was extracted from homogenized tumor tissue with TRIzol™ reagent (Gibco/BRL, Life Technologies Inc., Grand Island, NY, USA). Good RNA quality (RIN > 7) was assessed with a 2100 Bioanalyzer and a RNA6000 Nano LabChip kit (Agilent Technologies, Lexington, MA, USA). Affymetrix U133A GeneChips® were used to hybridize labeled targets prepared from total RNA (www.affymetrix.com). Hybridization assays and data collection were undertaken at the McGill University and Genome Quebec Innovation Centre as described at www.genomequebec.mcgill.ca/ovarian/. Affymetrix raw values were assigned by Affymetrix GeneChip software (MAS5) with an accompanying reliability score of present (P), marginal (M), and ambiguous (A). No GeneChips® used in this study had a >30% A score. Global normalization and preprocessing of the data were previously described in detail [34,35]. The dataset is described as in supplementary material, Table S1.

TMA and immunohistochemistry-supported histotyping

Areas of tumor were selected based on review of a H&E-stained slide. The FFPE tumor blocks for the

pilot set (EC and clear cell) were biopsied using a 1 mm diameter tissue arrayer, whereas the COEUR tumor blocks were biopsied using a 0.6 mm diameter arrayer. Tumor cores of both cohorts were assembled into a new tissue array, which was sectioned, stained with H&E, then stained for biomarkers and subjected to another pathology review to confirm tumor content and histotype [32]. On the pilot CHUM biobank EC cohort, 10 cases were reviewed as HGSC, one as the mucinous subtype and two without tumor content. On the COEUR cohort, 235 of 247 cases were confirmed EC. The TMAs were stained with antibodies routinely used in clinical pathology for diagnosis of ovarian tumors: SATB2 (clone EPNCIR130A, Abcam, Toronto, ON, Canada), CDX2 (EPR2764Y, Cell Marque, Rocklin, CA, USA) and PR (clone 1E2, Roche, Laval, QC, Canada). Normal colonic mucosa was used as a high expressor control while normal Müllerian tissue such as fallopian tube and endometrium were negative controls. Staining for each marker was performed on separate TMA slides using the Ventana automated immunostaining system. Antigen retrieval solution CC1 (#950-124, Ventana Medical System Inc., Laval, QC, Canada) was used for these antibodies. The immunohistochemistry-stained TMAs were scanned on the Olympus scanner and digitally stored for visual inspection. Scoring was performed by two observers. Nuclear SATB2 staining was evaluated as negative (0), weak (1), moderate (2) or strong (3) and by percentage of positive cells to calculate a combined score by multiplying the maximum intensity observed in a tissue core (range 0–3) and the percentage of positive cells. The range of possible scores was from 0 to 300. CDX2 and PR staining were evaluated as negative, focal (1–50%) or diffuse (>50%) for positive cells. β -catenin staining was evaluated as membrane or nuclear. Scoring was blinded to endpoint assessment.

Statistical analysis

Chi-square tests were used to assess the association between positivity for SATB2 and categorical variables. Pearson's test was used to estimate the correlation between SATB2 staining intensity and continuous variables (age of patients or age of samples). Multiple comparisons were controlled with the Bonferroni correction method. Analyses of DSS were evaluated with Kaplan–Meier curves coupled with the log-rank test. To estimate the hazard ratio (HR), we used Cox proportional regression in univariate and multivariate analyses with 95% CIs. Multivariate analyses were adjusted for age of patients, disease stage and source

Table 1. Clinical characteristics of the COEUR cohort and distribution of SATB2 immunostaining

	All (n = 231)	SATB2 negative (n = 176)	SATB2 positive (n = 55)	P value
Age diagnosis (mean, years)	57	58	53	0.006
Follow-up time (mean, months)	67	63	80	0.023
Overall survival rate	181/231 (78%)	132/176 (75%)	49/55 (89%)	0.027
DSS rate	191/231 (83%)	138/176 (78%)	53/55 (96%)	0.002
Progression rate	50/188 (26%)	43/143 (30%)	7/45 (16%)	0.055
Chemotherapy treatment				
Carboplatinum + taxol	137	100	37	
Cisplatinum + taxol	7	6	1	
Platinum alone	14	13	1	
Platinum + other	6	5	1	
No chemotherapy	25	121	40	
Total n	150	146	48	0.59
Stage				
I and II	178/218 (82%)	132 (80%)	46 (87%)	
III and IV	40/218 (18%)	33 (20%)	7 (13%)	
Total n	218	165	53	0.55
Residual disease				
Yes	35 (24%)	29 (26%)	6 (19%)	
No	110 (76%)	84 (74%)	26 (81%)	
Total n	145	113	32	0.86
Grade				
1	95	64	31	
2	79	59	20	
3	40	37	3	0.01
Morular metaplasia				
Yes	36	17	19	
No	208	171	37	
Total n	244	188	56	<0.01

Pearson's chi-square test for categorical variables and Pearson's correlation for continuous variables.

of samples; a second model was used with chemotherapy treatment and FIGO grade. An enter stepwise selection method was used for variable inclusion. A sample size of $n = 10k$ (n = number of events, k = number of variables) was considered before applying the multivariate analysis to avoid an overfit model. We assessed for proportional hazard assumption by visual examination of Kaplan–Meier plots and with the T-Cov function in SPSS software. Cases with missing data were not included in the analysis. Tests were two-sided. Significance level was set at $p < 0.05$ before the Bonferroni method. All statistical analyses were performed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA). The statistical analysis was carried out according to REMARK criteria.

Results

Expression of SATB2 in ovarian EC

To investigate the expression of SATB2 in OC, we first used a pilot set of 26 OC tissues of known histotypes, including two low-grade serous, three clear

cell, five EC and 16 HGSC (Figure 1A). Expression of SATB2 mRNA on the U133A Affymetrix microarray was detected in two EC cases: one with weak expression and one with strong expression (Figure 1A). CDX2 mRNA expression was not detected on this microchip, but strong expression of SATB2 mRNA correlated with upregulation of PGR mRNA.

The three clear cell carcinomas and the five ECs used in the GeneChips[®] assay had corresponding paraffin-embedded tissues available for a concomitant evaluation at the protein level. PR protein expression was more sensitive in immunohistochemistry than at the RNA level evaluated by RNA microarray (Figure 1). SATB2 mRNA expression strongly correlated with the protein staining intensity observed in EC tissues (Figure 1B). Furthermore, CDX2 and PR were present in tissues that were strongly positive for SATB2. Expression of SATB2 and of CDX2 was not specifically observed in tissues with morular metaplasia.

We did not observe any protein expression in the three clear cell carcinomas used in the GeneChips[®] assay or in a TMA containing 40 clear cell carcinomas stained by immunohistochemistry (see supplementary material, Figure S1).

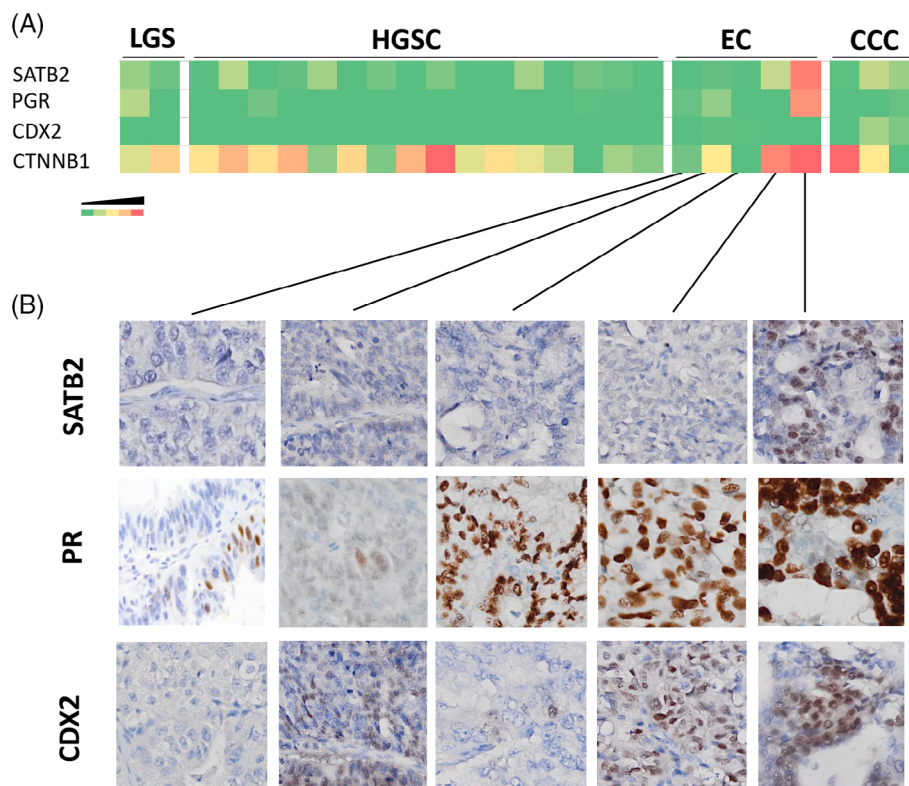


Figure 1. mRNA and protein expression of SATB2 in epithelial OC. (A) Affymetrix U133A microarray analysis of RNAs from epithelial OC ($n = 21$). Each column represents a sample and each row represents the expression of one gene. Color intensity represents gene expression levels. Green indicates lower than average expression, yellow equals average expression, and red represents higher than average expression. LGS: low-grade serous. CCC, clear cell carcinoma. (B) Representative staining for immunohistochemistry of SATB2, CDX2 and PR on EC tissue matching the cases analyzed on the U133A Affymetrix microchip. Magnification $\times 10$.

Correlation between nuclear SATB2 staining and clinical parameters

For evaluation of SATB2 expression in ovarian EC and its potential association with clinical parameters, we used the large EC TMA ($n = 235$) of the COEUR cohort. The characteristics of the tissue array are described in Table 1. Only six tissues were missing on the TMA and were re-evaluated individually on separate slides. Four cases had insufficient tumor content and could not be evaluated. With this TMA, a range of staining intensities with SATB2 was seen. Nuclear staining intensity was scored as weak, moderate and strong (Figure 2). A total of 26 cases (11.3%) demonstrated strong staining with percentage of positivity ranging from 1 to 90%. Moderate and weak staining was observed in 16 (6.9%) and 13 (5.6%) cases, respectively. The majority of positive cases ($n = 28$, 50%) showed an average of less than 10% of stained cells, and only three cases showed more than 50% of positive cells. The combined score ranged from 2.5 to 210. Only a weak correlation was seen between

staining intensity and percentage of positivity ($r = 0.23$, $p = 0.10$). We did not observe an exclusive expression of SATB2 in tissues associated with morular metaplasia (Figure 2), but a strong correlation (correlation coefficient $r = 0.29$, $p < 0.001$, Table 1). SATB2 was inversely correlated with age at diagnosis and rate of survival ($r = -0.18$ and $r = 0.20$, respectively; Pearson's test). No significant correlation was observed with clinical parameters such as disease stage, cytoreduction (Table 1), and previous or concomitant diagnosis of endometriosis or endometrial carcinoma ($p = 0.36$, $p = 0.39$, respectively; data not shown), with the exception of FIGO grade ($r = -0.20$, Table 1).

SATB2 association with patient survival

We performed Kaplan–Meier analysis and Cox proportional hazard models to estimate the association between SATB2 expression and patient survival. For this analysis, nuclear SATB2 staining was stratified into two categories: negative and positive (weak,

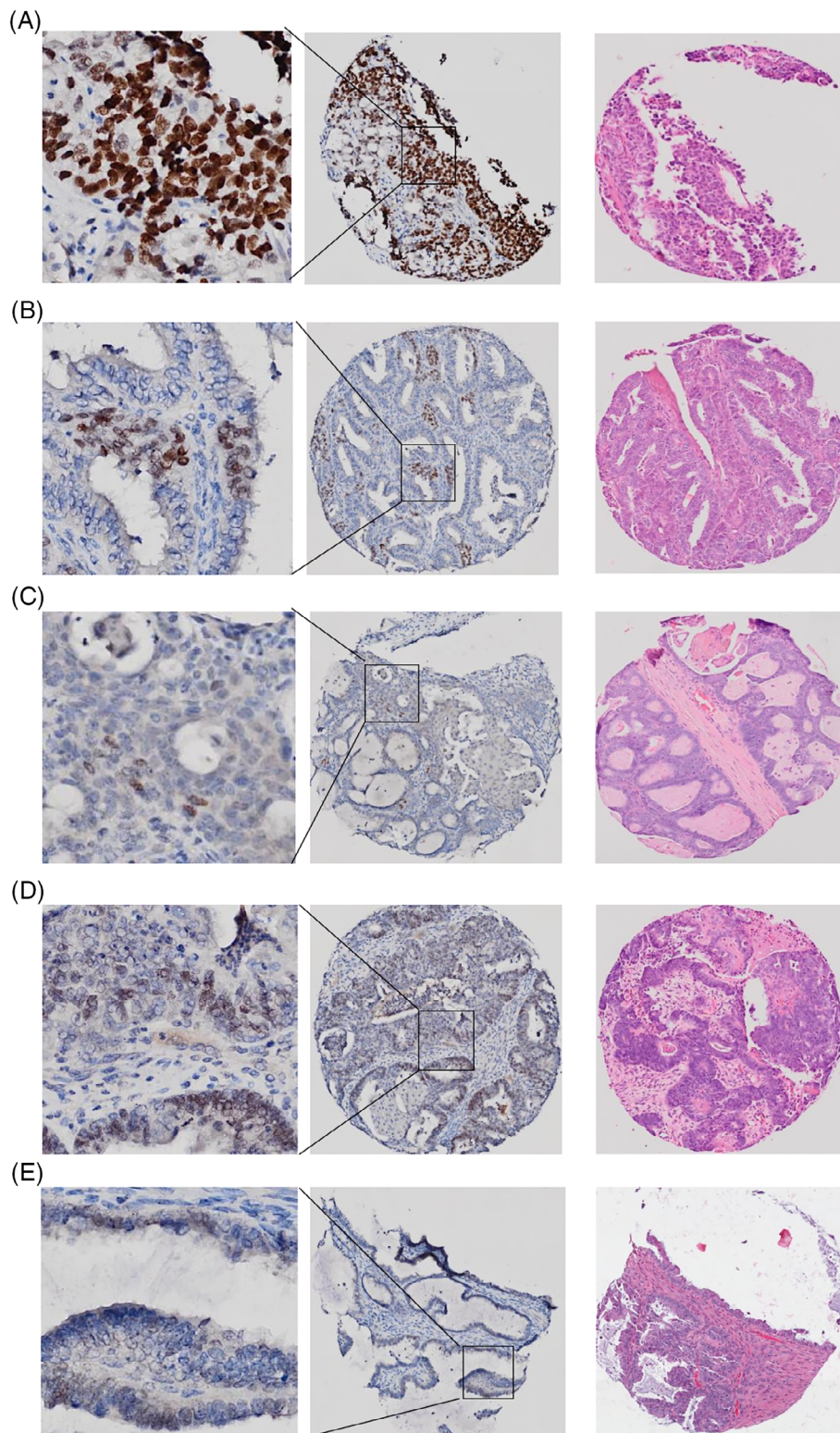


Figure 2. Representative immunohistochemistry staining of SATB2 and H&E from the COEUR Tissue Microarray. The two upper rows (A and B) show two cases without morular metaplasia and strong SATB2 staining (brown) and hematoxylin counterstain (blue). (C) Strong SATB2 staining intensity and morular metaplasia. (D) Moderate SATB2 staining. (E) Weak SATB2 staining. Left image is at magnification $\times 10$ of inset/boxed area in center image taken at magnification $\times 6.5$.

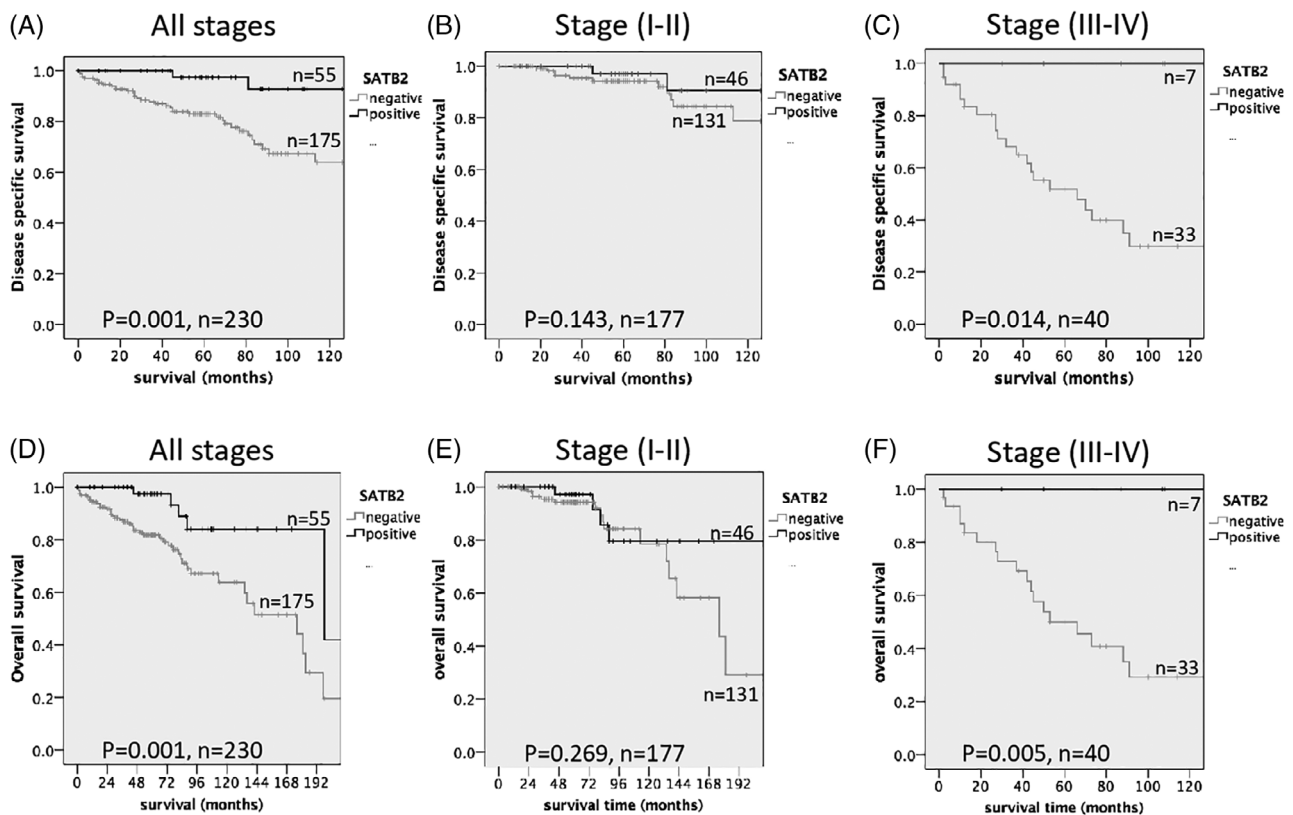


Figure 3. Kaplan–Meier survival analysis of SATB2 expression in the EC COEUR cohort. Analysis of disease-specific survival (A, B, and C) and OS (D, E, and F) in all patients (A, D), stage I and II patients (B, E) or stage III and IV patients (C, F). *n*: number of patients. *p*: *P* value (log-rank).

moderate, and strong intensity). As shown in Figure 3A and D, SATB2 positivity was associated with improved DSS and OS with HR = 0.14 (95% CI 0.03–0.56) and HR = 0.26 (95% CI 0.11–0.67), respectively (Table 2). Interestingly, the group of cases with positive SATB2 reached a survival rate of 96% (*n* = 53/55) compared to 78% (*n* = 138/176) in the negative group regardless of stage. In multivariate

analysis, SATB2 remained a strong prognostic factor, independent of patient age, stage or biobank source, with HR = 0.19, (95% CI 0.05–0.81) for DSS and HR = 0.31, (95% CI 0.12–0.83) for OS. While most patients were treated with standard carboplatin and taxol chemotherapy, adjustment for other chemotherapy regimens, including cisplatin/taxol, platinum alone or with another drug, did not impact the

Table 2. Univariate and multivariate Cox regression model of SATB2 on survival and disease progression

	Disease specific Survival		Overall survival			Progression		
	HR	<i>P</i> value	HR	<i>P</i> value	<i>n</i>	HR	<i>P</i> value	<i>n</i>
Univariate								
SATB2	0.14 (0.03–0.56)	0.006	0.26 (0.11–0.67)	0.005	225	0.42 (0.19–0.93)	0.033	187
PR (negative versus diffuse)	0.45 (0.22–0.88)	0.037	0.44 (0.22–0.88)	0.021	176	1.13 (0.53–2.41)	0.751	173
CDX2	0.49 (0.25–0.96)	0.038	0.63 (0.35–1.13)	0.119	220	0.37 (0.20–0.70)	0.002	179
β-catenin	0.69 (0.48–0.99)	0.044	0.74 (0.55–1.01)	0.058	222	0.73 (0.54–0.99)	0.05	182
Multivariate*								
SATB2	0.19 (0.05–0.81)	0.03	0.31 (0.12–0.83)	0.020	213	0.5 (0.22–1.14)	0.097	179
PR (negative versus diffuse)	1.02 (0.39–2.67)	0.974	0.95 (0.4–2.26)	0.901	165	1.39 (0.64–3.02)	0.409	166
CDX2	0.54 (0.24–1.22)	0.139	0.73 (0.36–1.46)	0.369	207	0.43 (0.22–0.86)	0.016	171
β-catenin	0.68 (0.44–1.07)	0.096	0.71 (0.48–1.05)	0.082	210	0.82 (0.58–1.14)	0.243	174

*Adjusted for patient age, disease stage and biobank source. HR, hazard ratio; *n*, number of patients.

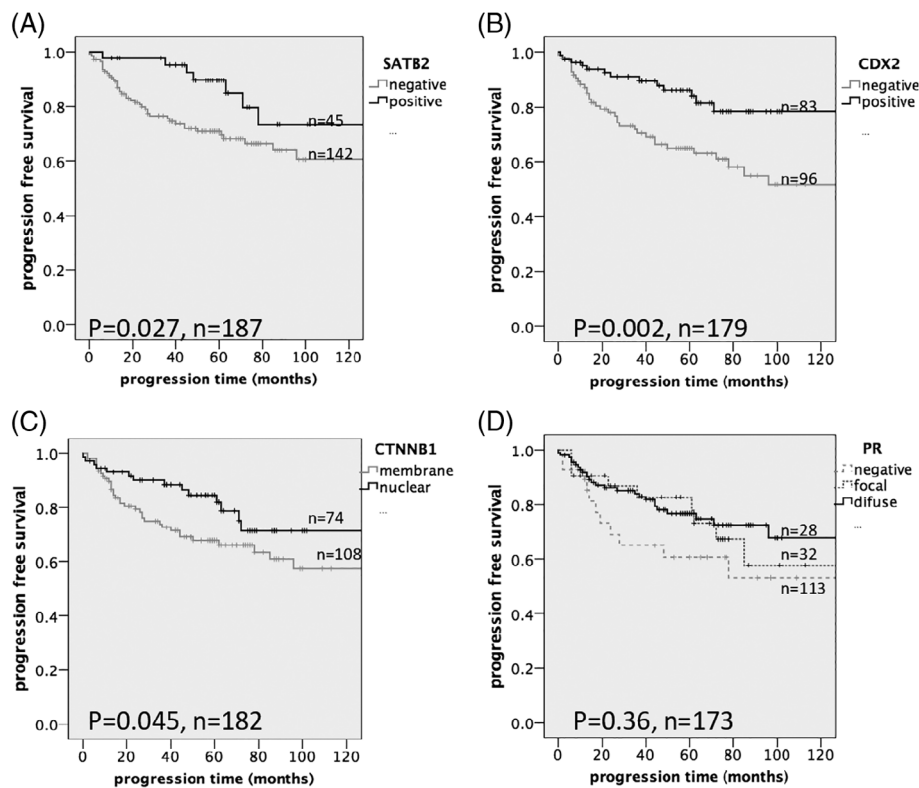


Figure 4. Kaplan–Meier progression-free survival analysis of SATB2 (A), CDX2 (B), β -catenin (CTNNB1) (C), and PR (D) in the EC COEUR cohort. *n*, number of patients. *p*: *P* value (log-rank).

association of SATB2 with survival (see supplementary material, Table S2). Although the cases treated with a regimen other than carboplatinum/taxol are rare, the evaluation of HR in these patients should be taken with precaution. Similarly, adjustment for FIGO grade did not reduce the HR of SATB2 (see supplementary material, Table S2). However, stratified analysis by disease stage showed that SATB2 was not significantly associated with DSS in a restricted group of patients diagnosed at stage I and II ($p = 0.143$ log-rank, Figure 3B) but was associated with DSS in stage III and IV patients ($p = 0.014$, log-rank, Figure 3C). Interestingly, disease-related deaths were not observed among patients diagnosed at stage III/IV with SATB2 expression. The limited number of cases in this group of patients did not allow a Cox regression analysis.

SATB2 association with disease progression

We then evaluated the association of SATB2 expression and disease progression. As shown in Table 1, a lower rate of progression was observed among SATB2-positive patients (30% versus 16%, $p = 0.055$, $n = 188$, Pearson's chi-square test). To confirm this

observation, we used Kaplan–Meier and Cox regression analysis. This also revealed longer progression-free survival in SATB2-positive cases compared to negative cases (Figure 4A) with HR = 0.42 (95% CI 0.19–0.93) and HR = 0.5 (95% CI 0.22–1.14) in univariate and multivariate analysis, respectively (Table 2).

Correlation between nuclear SATB2 staining and EC biomarkers

From the COEUR repository, we obtained immunohistochemistry data from other common diagnostic EC markers: CDX2, β -catenin, PR, estrogen receptor (ER), ARID1A and p16 (CDKN2A) [16,32,36–39]. These assays were performed on the same COEUR TMA. There was a significant correlation between SATB2 staining intensity and CDX2, β -catenin, PR, and ARID1A expression ($p < 0.001$, $p < 0.001$, $p = 0.041$, and $p = 0.05$, respectively; Pearson's chi-square test, Table 3). Most negative cases for SATB2 were also negative for CDX2 and expressed membrane β -catenin. Positive SATB2 cases were associated with focal expression of CDX2 and a diffuse expression of

Table 3. Distribution of SATB2 immunostaining with PR, CDX2, β -catenin, ER and ARID1A

	SATB2			P value
	Negative	Positive	Total	
PR				
Negative	28	4	32	
Focal	33	6	39	
Diffuse	99	42	141	
Total	160	52	212	0.041
CDX2				
Negative	104	14	120	
Focal	47	31	78	
Diffuse	17	7	24	
Total	168	52	222	<0.001*
β -catenin				
Negative	3	0	3	
Membrane	116	20	133	
Nuclear	55	34	88	
Total	174	54	224	0.001*
ER				
Negative	14	4	17	
Focal	11	1	11	
Diffuse	142	46	185	
Total	167	51	213	0.44
ARID1A				
Negative	35	18	53	
Positive	137	35	172	
Total	172	53	225	0.05
p16 (CDKN2A)				
Negative	13	4	17	
Heterogeneous	128	48	176	
Block	17	2	19	0.28

Pearson's chi-square test.

*P value significant with Bonferroni correction.

PR. A higher proportion of cases with negative expression (i.e. mutated) of ARID1A was also positive for SATB2. No correlation was observed between SATB2 expression and ER or p16 (Table 3).

SATB2 prognostic value compared to known prognostic biomarkers

As PR, p16, β -catenin and CDX2 biomarkers have also been associated with patient survival in ovarian EC [7,40–42], we questioned how the prognostic value of SATB2 compared with these markers. In the COEUR cohort, the prognostic value of p16 could not be validated due to the small number of cases with abnormal 'block' immunostaining. PR, CDX2, and β -catenin were individually associated with DSS and OS (see supplementary material, Figures S2A–S2C) but were not significant when adjusted for stage, age and sample source (Table 2), or for FIGO grade and chemotherapy (see supplementary material, Table S2). Among the four markers, SATB2 showed the strongest HR for DSS and OS in univariate and multivariate

analyses (Table 2). When restricted to early stage (I and II), CDX2 remained the only marker significantly associated with DSS ($p = 0.039$, log-rank, see supplementary material, Figure S2D–F). β -catenin and PR were not significantly associated with survival in patients stratified for stage (stage I and II or stage III and IV, see supplementary material, Figure S2). However, the number of cases was too small to evaluate CDX2 and β -catenin with confidence in advanced stage patients ($n < 40$, see supplementary material, Figure S2G–I).

Interestingly, CDX2 was also the most significant biomarker associated with disease progression (Figure 4A, D) (HR = 0.37, 95% CI 0.20–0.70, Table 2). More interestingly, CDX2 was also the only marker that was significantly associated with disease progression after adjusting for age and stage (HR = 0.43, 95% CI 0.22–0.86, Table 2).

Discussion

Here we report a frequency of SATB2 positive tumors (23%) that is higher than described in other studies or in the Human Protein Atlas. This discrepancy could be explained by the small number of EC cases reported in previous studies ($n < 20$). The EC histotype may have also been used without current marker-based diagnosis, and involuntary introduction of HGSC among EC may have contributed to the underestimation of SATB2 expression in ovarian EC. In contrast, the COEUR cohort has been rigorously reclassified for accurate histotyping [32]. In addition, only high and moderate expression levels of SATB2 are usually considered as positive in endometrial or gastrointestinal carcinoma. In our study, we also observed some cases with weak nuclear staining of SATB2 (Figure 2). This observation suggests that SATB2 expression in OC may have been underestimated, which is a potential pitfall since SATB2 is used to distinguish lower gastrointestinal metastasis to the ovary from primary OC.

Morular metaplasia in EC tissue is characterized by expression of CDX2 and nuclear β -catenin [36]. More recently SATB2 has been observed in tissue associated with morular metaplasia [15]. Although in this study a strong correlation between SATB2 and CDX2 or nuclear β -catenin was observed, exclusive expression of SATB2 in tissue with morular metaplasia was not observed. In line with our observations, Wang *et al* also observed an association of CDX2 or β -catenin with squamous differentiation in ovarian EC, but this was not always consistent [42]. In other words, these

markers can be expressed outside of morular metaplasia, although fewer than 50% of ECs are CDX2 positive (Table 3 and [42]). We also did not see a correlation between SATB2 expression and endometriosis ($p = 0.22$, Pearson's chi-square test) or in the clear cell carcinomas. Altogether these observations bring a better description of the SATB2 protein profile in OC. We only analyzed a small set of 18 HGSCs and we did not see SATB2 expression. If SATB2 is expressed in this tumor type at a lower frequency (<10%), it could have been missed. To firmly exclude that SATB2 is not expressed in the serous OC histotypes, a larger set of serous samples should be analyzed.

Interestingly, in the COEUR cohort, SATB2 was an independent marker of longer survival in ovarian EC even after adjustment for disease stage and patient age, or FIGO grade. When compared to other known prognostic markers, SATB2 was the strongest marker to predict OC-specific survival. A stronger association between SATB2 positivity and survival was obtained in advanced stage patients compared to early stage patients. Furthermore, SATB2 was also associated with lower rates of progression but only presented a trend when we evaluated the predictive value (HR) of SATB2 adjusted for stage and age. Altogether these data suggest that SATB2 is a promising biomarker that could complement the prognostic value of CDX2, PR, and β -catenin. A larger cohort would be necessary to validate this hypothesis.

Indeed, a limitation of our study is the low number of EC cases at advanced stage ($n = 40$) and with known progression status ($n = 140$). This drawback represents the demographic situation of this uncommon histotype of OC and the limitation of retrospective cohorts. The consequence of the small set of cases impacts the statistical significance of the tests and increased the confidence interval in Cox regression analysis. The data obtained here should be validated in other larger cohorts, although the availability of these cases is limited.

SATB2 and its association with improved outcome is reported here for the first time in OC; however, SATB2 is not new in other cancer types such as CRC, clear cell renal cell carcinoma and endometrial carcinomas (review in [30,31] and proteatlas.org). The mechanism by which SATB2 confers protection is not well defined. A study has linked SATB2 expression with down-regulation of MYC leading to a reduced cell proliferation rate in CRC cells [43,44], while another study in CRC correlated SATB2 expression with platinum sensitivity and improved survival time through an undefined molecular mechanism [21]. In

line with this hypothesis, SATB2 prognostic value is reduced in early stage EC patients who may not benefit from adjuvant chemotherapeutic treatment.

Generally, SATB2 is focally expressed in OC cells and present in less than 50% of the majority of tissues (Figure 2). In view of this expression profile and the strong protective effect on patient survival, we also hypothesize that SATB2 may have a paracrine role in ovarian EC. SATB2 may act through direct or indirect expression of cytokines, hormones or immunoregulators. Consistent with such a hypothesis is the observed correlation between SATB2 and PR expression in our cohort. In The Cancer Genome Atlas (TCGA) cohort, SATB2 mRNA expression also correlated with the expression of 3- β -hydroxysteroid dehydrogenase (HSD3B1) ($p = 0.05$, $r = 0.14$), the enzyme involved in the synthesis of progesterone. The validation of this paracrine hypothesis would require further molecular investigation.

In summary, the data from the COEUR multicenter cohort demonstrate for the first time that SATB2 expression is an independent marker of ovarian EC patient survival and potentially of disease progression. To our knowledge, there is no prognostic factor at this time that can help clinicians to predict long-term response to treatment or survival in advanced ovarian EC. The biologic role of SATB2 in ovarian cancer remains unknown and should be addressed in future studies.

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Author contributions statement

KR, MK contributed to immunohistochemistry analysis and provided histopathological diagnosis. KR, MK, CLP, and AMM-M contributed to the overall study design, drafting of the article, and interpretation of the findings. AMM-M provided reagents, materials and analytical tools. LM contributed to technical assistance. CLP contributed to statistical analysis. DMP contributed to tissue acquisition, critical revision of the manuscript for intellectual content. All authors gave final approval to the version submitted for publication.

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SUPPLEMENTARY MATERIAL ONLINE

Figure S1 Representative immunohistochemical staining for SATB2

Figure S2 Kaplan–Meier disease specific survival analysis of CDX2, β -catenin and PR

Table S1 Array data generated from Affymetrix U133A GeneChips corresponding to Figure A

Table S2 Univariate and multivariate Cox regression model of SATB2 on survival and disease recurrence adjusted for chemotherapy regimen and FIGO grade